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First report of fasciation symptom in *Artemisia eriantha* (*Asteraceae*), a typical orophyte of high-altitude cliffs, in Central Apennines (Italy)

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Abstract

Artemisia eriantha Ten., also known as "Apennines genepì", is a valuable aromatic plant with several medicinal properties. Among the several phytoplasma symptoms to which this plant can be subjected, the fasciation is one of the less reported and studied. In natural environments this plant is a characteristic species of the association *Artemisietum erianthae* Brullo 1984. In this study, the first occurrence of fasciation in *Artemisia eriantha* is described. This phenomenon was observed during the *ex vitro* experimental cultivations, located in Assergi and Barisciano (L'Aquila, Italy), settled for the restocking of this endangered species. The fasciation occurred only in Assergi experimental field with a magnitude of 0.12%. Specific PCR analysis did not show the phytoplasma presence. This fist report allows to expand knowledge about fasciation in Apennines genepì.

Keywords

Apennines genepì, *Artemisietum erianthae*, *Gran Sasso Monti della Laga* National Park, *Macrosiphoniella artemisiae*, micropropagation, restocking, stem fasciation

Introduction

Artemisia eriantha Ten. [syn. *A. umbelliformis* Lam. ssp. *eriantha* (Ten.) Vallès-Xirau and Brañas (Asteraceae)], known as "Apennines genepì", is a small plant of Central Italy showing 10-15 cm stems, bipinnate or tripinnate leaves, petioles and silvery-white trichomes. In natural environments its flowering period is in summer, and the flowers are yellow and organized in flower heads, arranged along the stem to form a spike (Abad et al. 2012). Regarding phytosociological aspects, *A. eriantha* is a characteristic species of the association *Artemisietum erianthae* Brullo 1984. The latter was described by Brullo (1984) in the high-altitude cliffs of Majella and Gran Sasso d'Italia, located above 2,200 m a.s.l. In view of the extremely precarious environmental conditions, this is a highly specialised cenosis. The author framed the association in the alliance *Saxifragion australis* Biondi and Ballelli ex Brullo 1984 [order *Potentilletalia caulescentis* Br.-Bl. in Br.-Bl. and Jenny 1926, class *Asplenietea trichomanis* (Br.-Bl. in Meier and Br.-Bl. 1934) Oberdorfer 1977]. This alliance brings together casmophytic and comophytic communities of the calcareous and dolomitic rocks. These communities develop in Temperate Macrobioclimate -in the thermotypes from Supratemperate to Superior orotemperate, sometimes with descents in the Mesotemperate in humid and fresh conditions (limestone gorges)- and in the Mediterranean Macrobioclimate -in the Supramediterranean to Oromediterranean thermotypes. It is widespread in the Central and Southern Apennines and in Eastern Sicily and can be considered, in these sectors, the vicariant of *Potentillion caulescentis* Br.-Bl. in Br.-Bl. and Jenny 1926 of Central Europe, Alps and Northern Apennines (Biondi et al. 2014). Biondi et al. (2000) indicate the species as the differential taxon of the subassociation *artemisietosum erianthae* Biondi, Allegrezza, Ballelli and Taffetani 2000 of the association *Caricetum kitaibelianae-rupestris* Biondi, Allegrezza, Ballelli and Taffetani 2000, described by

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the Authors for the Corno Grande of Gran Sasso d'Italia and belonging to the *Seslerion apenninae* alliance Bruno and Furnari 1966 (order *Seslerietalia tenuifoliae* Horvat 1930, class *Festuco-Seslerietea* Barbero and Bonin 1969). The association corresponds to a typical edafo-xerofilous formation in catenal connection with the *Elyna myosuroides*-dominated vegetation [*Elyna myosuroides* (Vill.) Fritsch, now *Carex myosuroides* Vill.], the so-called "elineti". This type of vegetation constitutes the Apenninic vicariant of *Caricetum rupestris* Pignatti and Pignatti 1984 of the Italian Dolomites (Pignatti and Pignatti 1985). The subassociation describes the communities established on the microterraces and microdepressions of the northern rocky crests.

A. eriantha is also present, sporadically or with low cover values, in other vegetation types of high altitude, for example, in the associations *Saxifrago speciosae-Silenetum caenisiae* Petriccione 1993, found on Sibillini (Costanzo et al. 2009); *Potentillo apenninae-Festucetum alfredianae* Blasi, Di Pietro, Fortini and Catonica 2003 (*Saxifragion australis* Biondi and Ballelli ex Brullo 1984) found on Gran Sasso (Blasi et al. 2003); and *Saxifrago speciosae-Papaveretum julici* Feoli Chiapella and Feoli 1977 found on Majella (Blasi et al. 2005).

This aromatic plant produces oils with a complex terpenoid profile, rich in sesquiterpenes and several compounds and whose content increases with the plant age (D'Andrea et al. 2003; Reale et al. 2011; Reale et al. 2014). For wild and micropropagated plants of this species the thujones have been recorded as major constituents (60–85%), with the predominance of the α isomer (Pace et al. 2010). Beyond its strong scent, exploited as flavouring agent, Apennines genepì is well-known for its medicinal properties. Its namesake liqueur and infusions, in fact, are utilized in traditional medicine for their thermogenic properties and are recommended to counteract airways infections, weakness and indigestion (Appendino et al. 1982; Mucciarelli and Maffei 2002; Simonnet et al. 2006; Rubiolo et al. 2009; Maggio et al. 2012; Vouillamoz et al. 2015).

Like other medicinal and aromatic plants, Apennines genepì can be affected by several symptoms. Among them, fasciation is one of the most interesting phenomena, in fact, it is distinct from other kinds of deformity (Choob and Sinyushin 2012). It may arise because of (i) genetic mutations, with a progeny that inherits the changed phenotype (Samineni et al. 2008; Iliev and Kitin 2011; Onda et al. 2011) or (ii) in response to abiotic (e.g. cytokinins, herbicides, photoperiod, radiations - Thimann and Sachs 1966; Jambhulkar 2002; Chriqui 2008; Bondada 2011) and biotic (e.g. *Rhodococcus fascians*, *Candidatus Phytoplasma* spp. or other pathogenic bacteria - Battikhi 2002; Putnam and Miller 2007; Bertaccini and Duduk 2009; Wu et al. 2012; Akhtar et al. 2013; Pavlovic et al. 2013; Yu et al. 2016; Gahukar 2018) factors, not transmitting this altered state to their progeny.

Fasciation is very common in species belonging to several families (e.g. Asteraceae, Rosaceae, Ranunculaceae, Liliaceae, Euphorbiaceae, Crassulaceae, Fabaceae, Onagraceae and Cactaceae – White 1948; Binggeli 1990; Kumar et al. 2010), however, many interesting features of fasciation development still have no explanations (Iliev and Kitin 2011). In addition, information about the frequency of this phenomenon is also very limited and many reports on fasciated plants do not describe the inducing agent.

In the study area, cultivation practices of Apennines genepì have been proposed to restock this endangered species and to offer the local populations the opportunity to promote economic activities in high altitude areas, whilst preserving their local habits and traditions (Pace et al. 2010; Di Cecco et al. 2017; Chelli et al. 2019). In this study we describe a fasciation in Apennines genepì propagated *in vitro* and transplanted in experimental fields.

Materials and methods

Plant material and micropropagation

In vitro Apennines genepì plantlets and tissue cultures were obtained from seeds collected on Monte Portella 2500 m asl (*Gran Sasso Monti della Laga* National Park) in August 2013 (Fig.1a). Seed surface sterilization and *in vitro* germination were obtained according to the method previously described in Pace et al. (2004). Rooted plantlets were transferred to soil and kept in a greenhouse as previously described (Pace et al. 2020) before being transplanted to the experimental fields.

Experimental fields

In spring 2014 about 10.000 clones were obtained by *in vitro* propagation and at the end of May they were transplanted in two experimental fields located at Barisciano (AQ) and Assergi (AQ) in the *Gran Sasso Monti della Laga* National Park. The main features of selected sites are detailed in Table 1 (soil pH, potassium, phosphorus, and nitrogen were measured according to standard methods by an external laboratory).

Plant fasciation

At the end of May 2015, the adult plants were mapped and collected; during the samplings twelve fasciated plants were discovered at Assergi experimental field, randomly spread throughout the field. The plants were observed and photographed then were brought in laboratory. After that the width of the stems just below the fasciated heads was measured and the number of flower heads was counted. Two sample T-test was used to evaluate whether there was a significant difference between fasciated and non-fasciated plants with "Past3" software for experimental field of Assergi. Some plants were dried, others were cryopre-

Table 1. Features of field sites of *Artemisia eriantha*.

Parameter	Barisciano (AQ)	Assergi (AQ) San Pietro Camarda
Altitude (m)	1170	1200
Geographical coordinates	42°20'25"N, 13°34'14"E (DMS)	42°26'30"N, 13°28'19"E (DMS)
Fertilization	None	None
Previous cultivation	uncultured	*Solanum tuberosum*
Soil pH	8.35	8.35
P (g/kg)	6	9 478
N	1.65	2.16
K	6	9.47

served at -80°C and were used for nucleic acid extraction (Prince et al. 1993) for phytoplasma detection by PCR amplification using nested system with primers amplifying the 16S ribosomal gene (Gundersen and Lee 1996).

Aphids identification

Some specimens of nymphs and adults of aphids observed in mid May 2015 at Assergi experimental field, were collected in plastic bags and brought to the laboratory. According to the current preparation method for aphids (Blackman and Eastop 2000) they were identified by specialists to species level.

Results

A. eriantha specimens did not show any abnormality during micropropagation process. Although transplanted clones exhibited a high morphological plasticity, with different sizes of rosette and various number of shoots, they were morphologically identical in the two experimental fields ($p > 0.05$). In 2015 spring, the flowering was exceptionally abundant up to 16 flower-heads for rosette (Fig. 1b).

Except for mineral contents, soil analysis did not show differences between the two experimental fields. The highest mineral content was recorded for Assergi field and was due to the previous cultivation with *Solanum tuberosum* L. (Table 1).

In Table 2 the biometrical parameters (i.e. number of flower heads and width of stems) are reported, recorded for the twelve fasciated (Fig.1c) and normal plants collected from Assergi experimental field and both fields, respectively. As shown, the fasciated plants had significantly higher mean number of flower heads ($p < 0.001$) than those counted on the normal plants. The width of the stems was also significantly higher in fasciated plants ($p < 0.001$) than those reported for the normal ones.

The nested PCR tests carried out on nucleic acid extracted from symptomatic materials provided negative results for phytoplasma presence.

The occurrence of aphids was observed only on upper parts of Apennines genepì of Assergi experimental field. The infestation was observed especially between inflorescences, and both adults and nymphs were found (Fig.1d). The identification allowed to establish that aphids (Fig.1d) belong to the species *Macrosiphoniella artemisiae* (Boyer de Fonscolombe 1841). It is a Palearctic species widespread from North Africa and Europe to Siberia, Mongolia, and China, which was accidentally introduced to North America (Blackman and Eastop 2007). Very common in Italy on *Artemisia vulgaris* (typical feeding plant for this aphid), it is found among the inflorescences as large colonies (Barbagallo et al. 2011, Barbagallo and Cocuzza 2014). It can occasionally be found also on *Leucanthemum vulgare*, *Tanacetum parthenium* and other species of *Artemisia* (Mifsud et al. 2011).

Discussion

As stated before, the biotic factors can have an inducing ability on fasciation arising (Battikhi 2002; Bertaccini and Duduk 2009; Wu et al. 2012; Akhtar et al. 2013; Pavlovic et al. 2013; Yu et al. 2016). PCR analysis did not show the presence of phytoplasma, thus, in the current study fasciation is not associated with the presence of these pleomorphic bacteria. On the contrary, the presence of *M. artemisiae* was observed only on upper parts of Apennines genepì of Assergi experimental field. The presence of *M. artemisiae* on *A. eriantha* is reported for the first time in this study; however, this phenomenon cannot be correlated to fasciation of plants. The aphids, in fact, were also found on plants without symptoms of the same field. The crop previously cultivated on Assergi experimental field was *Solanum tuberosum*, and Avila et al. (2014) reported the presence of this aphid species on potatoes cultivations. Thus, we cannot exclude that the presence of the aphid on Apennines genepì can derive from an unappropriated field management strategy, that left this infestation on the field.

Fasciation was noted only in the field of Assergi. Both the fields were transplanted with plantlets derived from clones obtained from several seeds. Thus, this phenomenon could result also from micropropagation process. Multiplication of plants by this technique, can induce phenotypic aberrations in *ex vitro* cultivations that were not observed during *in vitro* culturing (Shirani et al. 2009; Iliev and Kitin 2011; Chiruvella et al. 2014).

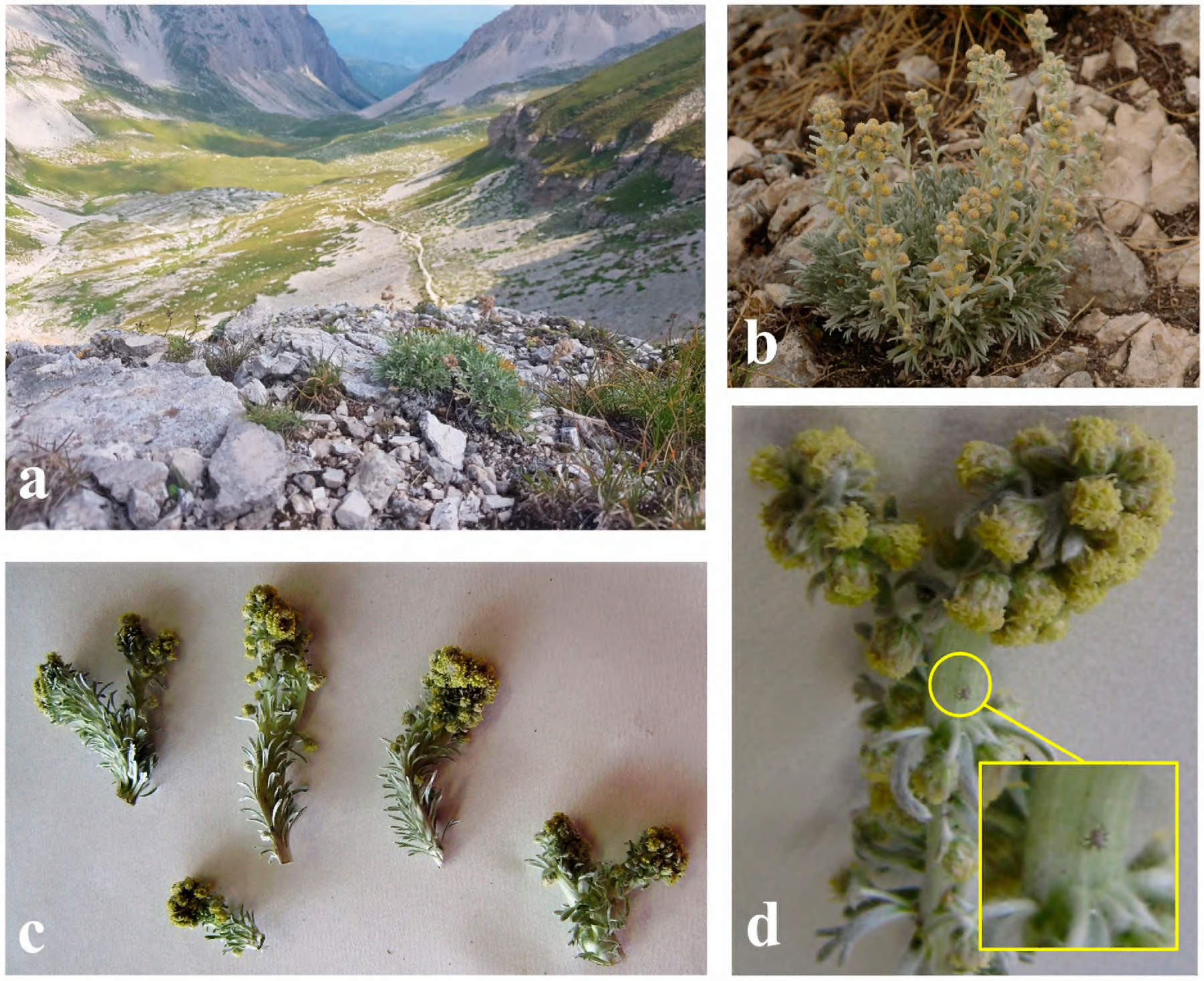

Figure 1. a) One of the natural stations on Gran Sasso massif. b) Rosette of *ex vitro Artemisia eriantha* photographed on experimental fields. c) *A. eriantha* fasciated stems and inflorescences. d) *Macrosiphoniella artemisiae* adults on fasciated plants.

Table 2. Biometrical parameters recorded for fasciated and normal plants of *Artemisia eriantha* (mean±SD).

Sample	Flower heads	Width of stems
Fasciated plants	23.58 ± 7.29	7.74 ± 6.54
Normal plants	16.74 ± 5.26	1.15 ± 0.254
T-test	t = 4.40; $p < 0.001$	t = 22; $p < 0.001$

Conclusions

Although micropropagation process can be a valid tool for the recovery of plants threatened by climatic change and undiscerning collection, this technique might induce phenotypic aberrations in *ex vitro* cultivations and should be for this reason used with great care. This process, together with the presence of other biotic and abiotic factors, could have induced plant fasciation in *Artemisia eriantha*. The presence of the aphid *Macrosiphoniella artemisiae* on Apennines genepì is here described for the first time and shows that careful control of aphids' infestation, by appropriate field management strategies, is important to protect the restocking of this aromatic plant.

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